The role of Estrogen, in Leukocytes, Mitochondrial, along with Cytokine Function and Regulation, and Cancer and Autoimmune Diseases Treatment and Prevention

Dr. Zelalem Kiros Bitsue, Dr. Zahraa Nashee hamad Almohammed,
Prof. Iraj ragerdi-Kashani, Prof. Parichehr Pasbakhsh

United States of Africa Health Organization "AHO",
Department of Immunology, International Campus, Tehran University of Medical Sciences
Department of Anatomy, International Campus, Tehran University of Medical Sciences,

Abstract

Estrogen is active both on vascular smooth muscle and endothelial cells where functionally competent estrogen receptors have been identified. Traditionally, the actions of 17β estradiol are ascribed to two nuclear estrogen receptors (ERs), ERα and ERβ, which function as ligand-activated transcription factors.

Recent evidence demonstrates the presence of estrogen receptor in various cell types of the immune system, while divergent effects of estrogens on the cytokine regulation are thought to be implicated.

Estrogen administration promotes vasodilation in humans and in experimental animals, in part by stimulating prostacyclin and nitric oxide synthesis, as well as by decreasing the production of vasoconstrictor agents such as cyclooxygenase- derived products, reactive oxygen species, angiotensin II, and endothelin-1.

In vitro, estrogen exerts a direct inhibitory effect on smooth muscle by activating potassium efflux and by inhibiting calcium influx. In vivo, 17ß-estradiol prevents neointimal thickening after balloon injury and also ameliorates the lesions occurring in atherosclerotic conditions. Most recently, estriol has shown the potential to treat individuals with Th1-mediated autoimmune illnesses, including multiple sclerosis and rheumatoid arthritis.

This article will update the clinical effects and the role of Estrogen, in Leukocytes, in Mitochondrial, Actions of estrogen on endothelial cells, Cytokine Function and Regulation, and further clarify the documented advances which support the substantial therapeutic benefits of Estrogen for Cancer and autoimmune conditions.

Key Word: Estrogen, Mitochondrial, Cytokine, Leukocytes, Immunoregulatory and Immunomodulatory, Cancer, and Autoimmunity

The table of contents

1. Introduction

17β-Estradiol is commonly recognized as the predominant female sex hormone, and function in males.1 In addition to the reproductive system, 17β-estradiol has important physiological roles in almost every other area of the body, including the nervous, immune, vascular, muscular, skeletal and endocrine systems. As expected, disruptions in 17β-estradiol signaling, therefore, contribute to multiple disorders, including cancer, cardiovascular diseases, hypertension, osteoporosis, cognitive and behavioral alterations, neurodegenerative diseases, metabolic disorders (such as obesity and diabetes mellitus) and immune disorders (1). Our understanding of the widespread physiological effects of 17β-estradiol is complicated by the existence of several types of estrogen receptors (ERs) and multiple modes of cellular signaling mechanisms that span time frames from seconds to hours, or even days (2),(3). The pathophysiological mechanisms involving ERs are further complicated by a diverse array of 17β-estradiol-

mimicking compounds, both synthetic and plant-derived, to which humans are increasingly exposed (4).

Recently a membrane bound G protein-coupled receptor-30, now designated as G protein-coupled estrogen receptor- 1(GPER), has been described as a receptor for estrogen (5). ERα and ERβ belong to the nuclear receptor superfamily and functions as ligand activated transcriptional factors. The classical mechanism of nuclear ER action involves ligand binding to receptors, dimerization and binding to specific response elements of the target genes to elicit a transcriptional response.

Estrogens can also act rapidly through nongenomicmechanisms by binding to membrane bound ERs (1), (5). GPER is a member of the G protein-coupled receptor superfamily containing seven transmembrane helices and mediates estrogen-dependent kinase activation as well astranscriptional responses (5). Receptors for estrogens are present in leukocytes and perform various functions (6). ERs are present in a variety of leukocytes like myeloid progenitor cells, neutrophils, lymphocytes, natural killer cells, macrophages, monocytes, mast cells etc.

2. Estrogen receptors

2.1. ERα and ERβ

The first and best described 17β-estradiol receptor, now called ERα, was identified in the rat uterus in the 1960s (7),(8). The second, less well-characterized receptor, ERβ, was identified in the rat prostate in 1996 (9). These highly homologous receptors function as ligand-activated nuclear transcription factors that bind *cis*-acting estrogen response elements in the promoter and enhancer regions of hormonally regulated genes (10). Both ERα and ERβ, encoded by the genes *ESR1* and *ESR2*, respectively, are soluble receptors that can shuttle between the cytoplasm and the nucleus, but are found predominantly in the nucleus (only ~5% of these receptors are present in the cytoplasm).4 Highly divergent and sometimes opposing functions for the two receptors have been reported in studies of *Esr1* knockout and *Esr2* knockout mice, which lack the murine ERα and ERβ protein, respectively (11). In addition to their effects on gene expression (that is, their genomic effects), these ERs are also associated with rapid cellular signaling (termed non-genomic effects) that are thought to be mediated primarily by membrane-associated forms of these receptors (12). Although multiple modes of action were suggested for ERs as early as the 1960s (13),(14),(15), not all effects of 17β-estradiol, particularly the rapid and membrane-associated signaling cases, antagonists of these receptors could not block certain rapid signaling events, which led to the prediction that alternative membrane-bound ERs also existed (16). Interestingly, most of the 17β-estradiol-mediated rapid signaling

2.1.2. GPER

A study in 2000 reported that rapid 17β-estradiol-mediated activation of extracellular signal-regulated kinases (ERKs) was dependent on the expression of an orphan G-protein-coupled receptor with seven transmembrane domains (17). This receptor, then known as GPR30, was cloned by several groups in the late 1990s (18),(19),(20),(21),(22),(23). Following this initial report, other studies described 17β-estradiol-mediated, GPR30-dependent, generation of

cAMP(24), 24 and expression of Bcl-2, (25). nerve growth factor (26), and cyclin D2 (27). Furthermore, other researchers described GPR30-mediated expression of c-Fos (28), and an interaction between the effects of progestin and GPR30 expression (29),(30),(31). Two studies published in 2005 described binding of 17β-estradiol to GPR30 in GPR30-transfected COS7 and HEK293 cells, as well as various breast cancer cell lines (32),(33). Together, these results suggested that GPR30 was a 17β-estradiol-binding receptor, which led to its designation as G-protein-coupled estrogen receptor 1 (GPER) in 2007. GPER is now known to be expressed in numerous tissues,(34), and research into its functions has substantially increased.

3. Estrogen receptor ligands

3.1. GPER unselective ligands

Natural endogenous estrogens, predominantly 17β- estradiol, are the primary ligands of ERs. 17β-estradiol is synthesized mainly in the ovaries, although it is also produced at many sites throughout the body, including the breast, brain, adipose tissue and the arterial wall, where it might have specialized local effects (35). The 17β-estradiol-based steroids estriol (a GPER antagonist at high concentrations (36), estrone and estrone sulfate can also modulate biological functions, although their specific actions are less clear than those of 17β-estradiol.(37). Plasma concentrations of 17β-estradiol in premenopausal women are ~0.2– 1.0 nmol/l, although it increases by many 100-fold during pregnancy. Local concentrations in specific tissues can be much higher than the plasma values, for example in breast tissue (by 10–20-fold) (38), or in the placenta at term (~12 μmol/l) (39). The hydrophobic nature of these steroids allows them to diffuse passively through cell membranes and reach their intracellular targets, the ERs (40).

A large variety of natural and man-made chemicals also have estrogenic activity. Estrogenic compounds synthesized by plants (phytoestrogens) include flavonoids, such as coumestans and isoflavones (41). Synthetic estrogenic compounds (known as xenoestrogens, environmental estrogens or endocrine disruptors) include many pesticides, herbicides and plastic monomers. Their widespread use results in chronic low-level exposure to these compounds in humans (42). Although the majority of phytoestrogens and xenoestrogens are believed to exert their physiological effects through modulation of ERα and ERβ,(43), many of these compounds also activate GPER, including the soy isoflavone genistein, for which serum concentrations up to 500 nmol/l have been measured;(44), nonylphenol; the pesticides dichlorodiphenyltrichloroethane (DDT) and dichlorodiphenyldichloroethylene (DDE); bisphenols,(45),such as bisphenol A (Figure 1), which promotes testicular seminoma cell proliferation (46), the herbicide atrazine; (47), and possibly equol, a nonsteroidal equine estrogen found in premarin48 that is formed by human gut bacteria as a metabolite of the isoflavone, daidzein.(48).

Synthetic 17β-estradiol mimetics are also used extensively in clinical and therapeutic applications. For example, 17α-ethynylestradiol is the predominant estrogen used in female contraceptives. Drugs, such as tamoxifen and raloxifene, which are used in treatments for breast cancer and osteoporosis,2 act as ER agonists in some tissues and ER antagonists in others, which led to their designation as selective estrogen receptor modulators (SERMs) (49). By contrast, fulvestrant is a ‘pure’ ER antagonist that causes ER degradation and/or downregulation, which led to its designation as a selective estrogen receptor downregulator (SERD) (50). However, some members of SERMs and SERDs can also act as GPER agonists,17,33 which complicates the interpretation of the mechanisms of their action and the receptors involved in both physiological and disease conditions (51).

3.1.2. GPER-selective ligands

Research into the specific activities of GPER has been aided by the discovery of GPER-selective agents. Since the identification of the first GPER-selective agonist G-1 in 2006, a number of reports have examined the disease-related or health-promoting effects associated with GPER activation. Importantly, studies using G-1 at concentrations as high as 1–10 μmol/l showed no notable activity of this agent towards ERα in terms of activating or inhibiting rapid signaling events,(33). estrogen response element-mediated transcription (52),(53), or ERα downregulation (52). Furthermore, G-1 had no binding activity on 25 other G-protein-coupled receptors (54), or in *Gper* knockout mice,56–58 which provided evidence that G-1 is a ligand highly selective for GPER.
In 2009, the GPER-selective antagonist G15 was identified, (55). followed by G36, a more selective GPER antagonist than G15, identified in 2011.(53). G15 has a similar structure to G-1,(55). and is effective in inhibiting all G-1-mediated effects tested to date,(55),(56),(57) as well as many 17β-estradiol-mediated effects.(55), (56),(57),(58),(59). The core structures of G-1, G15 and G36 have been used to generate several radioactively labeled agents that can be used for imaging and potential treatment of GPER-expressing tumors *in vivo* (60),(61).

4. Physiology y of Estrogens

Estrogens are steroid hormones present in both men and women, but present at significantly higher levels in women of reproductive age. There are three naturally occurring estrogens in women: estrone (E1), E2, and E3 (62). The developing follicles in the ovaries are responsible for producing the majority of the estrogens. There is also evidence that some estrogens are formed by the liver, adrenal glands, muscle tissue and fat cells through conversion of C19 precursors to C18 steroids by aromatase (63). Adipose tissue expression of aromatase increases with body weight and increasing age, thus is of concern in in post-menopausal women.9 Synthesis of estrogens occurs in the theca interna cells of the developing ova, and is accomplished through conversion of androstenedione from cholesterol (64),(65). E2 can be converted to E1 and E3. E1 can be converted to E2 or E3. However, E3 is not interconvertible and hence, does not result in an increase of either of the other two estrogens.

5. Estrogen Signaling and the Regulation of Cytokines in Immune Cells

Given the central role of estrogens in stimulating SLE disease and because cytokines are substantially involved in the pathogenesis of SLE, we herein provide evidence elucidating the molecular basis of the interplay between estrogen and cytokines in immune cells/organs known to be of crucial importance in the control of the autoimmune response. Because estrogensmediate their effects via estrogen receptors (nuclear isoforms and/or membrane receptor), studies have focused on the detection of the above receptors in the immune cells (B cell, T cells, dendritic/macrophages, monocytes) as well as in the immune organs (thymus) and their possible role in autoimmunity(66). The thymus is an immune organ of prime importance since it is well known that CD4+ and CD8+ T cell development is a result of a complex process, starting with the migration of progenitors from bone marrow to the thymus and followed by positive and negative selection processes that are critical for both final maturation of T cells and

prevention of autoreactivity(67). The loss of thymus function, after ablation of a hyperplastic thymus has been shown to contribute to the development of SLE(68). Moreover, the MRL / lpr strain (an SLE mouse model) develops early in life autoimmune diseases characterized by thymic atrophy(69). Firstly, Stimson and Hunter(70), demonstrated estrogen receptors in the human thymocytes. Estrogen has been shown to lead to thymic atrophy through various mechanisms including, among others, modulation of the production of IL-7, an important regulator of Tlymphopoiesis (71). It is of import to bear in mind, the presence of both ERα and ERβ is required to exert this action, while in mice it has been demonstrated that ERα needs to be expressed in both the hematopoietic and stromal compartments of the thymus (72); on the other hand, the achievement of a full-sized thymus requires the presence of ERα in stromal but not in thymic cells (72). Additionally to the T cell development, estrogen has been shown to exert important effects on T cell function through ERs which have been identified in both CD4+ and CD8+ T cells (73),(74), in a biphasic way. As already mentioned, that SLE is characterized by a shift from the balance between Th- 1 and Th-2 subsets to Th-2 dominance. It is well known that low doses of estrogen promote enhanced Th-1 responses and increased cell-mediated immunity, while high doses of estrogen lead instead to increased Th-2 responses (75),(76). Of note, the enhancement of Th-1 responses to low-dose estrogen required the presence of ERα, but not ERβ (75). The high estrogen levels that accompany pregnancy may account for the stronger humoral responses and possibly contribute to the flares that some SLE patients experience during pregnancy (77),(78). This effect of estrogens seems to be achieved through direct alteration in the Th cytokine profile from a proinflammatory (IL-2, IFN-γ, TNF-a) to an anti-inflammatory direction (IL-4, IL-6, IL-10, TGF-β) (79). Indeed, E2 as well as estrone and estriol have been shown to stimulate IL-10 in human CD4+ cells (80),(81), while their effect on antigen-stimulated secretion of TNF-a was biphasic, with enhancement at low concentrations and inhibition at high concentrations (80). A stimulatory effect of estriol on IL-10 production, in contrast to the inhibitory effect on TNF-a, has also been revealed by Zang and coworkers (82).

Lambert et al (83), in agreement with previous studies (84),(85), observed a significant E2 induction of IL-4 secretion by purified CD4+ T cells, an effect mediated through ERα in a classical ligand-dependent manner. IL-2, another cytokine important for differentiation of T cell responses into Th-1 or Th-2 predominance, has been found to be suppressed by high concentrations of estradiol in activated peripheral blood T cells and CD4+ T cell lines (86).

A recent and highly interesting study by Xia et al.(87), showed that estrogen replacement therapy increased the IL- 4 while decreasing IL-2 and IFN-γ secretion by T cells isolated from surgically induced menopausal women, an effect which seems to be mediated mainly through ERα. An ERα-mediated regulation of IL-2 and IFN-γ secretion is also exhibited in splenic T cells (84) Other cytokines, like IL- 12 which is a central stimulator of Th1 type cytokines(88), as well as IL-1, are also influenced by estrogen action in T cells, with divergent results (76),(89). IL-17 is a novel cytokine derived from T cells which has been shown to play an important role in the Th-1 / Th-2 balance(90), and has recently been implicated in the pathogenesis of SLE(91).

A recent study showed that estradiol reduces the production of IL-17 by upregulation of PD-1 (programmed death 1) expression within the Treg-cell compartment, an effect mediated through membrane ER(92),(92). With respect to IL-6, most studies, albeit conducted in PBMC or whole blood cultures which include multiple cellular components, demonstrated an inhibitory effect of estrogen(93),(94). Many studies have shown that estrogens regulate the cytokine gene expression in different cell types, via ERmediated pathways, either directly through EREs or indirectly through interaction of ER with other transcription factors including NF-kB and AP-1.

EREs have been recognized in the promoter of IFN-γ and IL-10 genes(95),(96). It is of note that NF-kB response elements have also been found in the promoter of several cytokine genes like IL-6, IL-10, TNF-a IL-1β, IL-12, and IL-2 (97),(98),(99), while there are cytokines, that can be transcriptionally regulated through interaction of more than one transcription factor. Indeed, IL-2 is tightly controlled by several transcription factors that bind to the IL-2 promoter; these transcription enhancers include NFAT, NF-kB, and AP-1, while there is evidence that all binding sites on the IL-2 promoter need to be occupied to ensure maximal transcritption and production of IL-2(99),(100). Disturbances in the transcriptionally active proteins, including NFAT (nuclear factor of activated T cells), members of the AP-1 (c-Fos, c- Jun), and NF-kB (c-rel, p50, p65) families, have been shown to modulate cytokine gene expression in activated T cells (97),(98),(99),(99),(100),(101),(102). Importantly, abnormal NF-kB signaling characterized by decreased p65-RelA, increased c-rel and reduced NF-kB DNA binding activity, associated with altered cytokine expression, has been recognized in T cells from SLE patients (103),(104) ,(105),(106),(107). Similarly, disturbances in AP-1-signaling associated with alterations in c-fos protein expression and AP-1 DNA binding activity has also been demonstrated in T cells from SLE patients (107),(108),(109). The question now arises: how the estrogen-estrogen receptor pathway cross-react with NF-kB and / or AP-1 mediated signaling in lymphocytes to regulate cytokine gene expression? Mechanistic studies on the direct interaction between ER and NF-kB / AP-1 in immune cells, which is presumed important in the control of estrogen-induced immune responses and cytokine expression, are only sparse. Zang et al. were the first to reveal that estriol altered cytokine profile of human normal T cells through inhibition of IkBa degradation; interestingly, this effect was specific for NF-kB and not for other transcription factors like AP-1, and was ER-mediated, since it was partially abolished in the presence of tamoxifen (109),(110). However, a recent study has demonstrated that E2, acting through ERβ, could directly enhance NF-kB activity in human T cells, suggesting that estrogen actions on NF-kB activity may depend on ER and cell subtypes (111). An enhanced transcriptional activity of nuclear NF-kB p65 in macrophages from E2-treated mice was also observed by Calippe et al.(112), according to their results, this enhanced NF-kB activation could be a consequence of the reduced PI3K activity, as a result of chronic exposure to estrogens, through direct or indirect mechanisms, a hypothesis, however, which remains to be elucidated.

Apart from T-cells, monocyte-derived dendritic cells are substantially involved in the pathogenesis in SLE. Dysfunctional dendritic cells in human SLE have been described. In studies describing dysfunctional dendritic cells in human SLE(113),(114), monocyte-derived dendritic cells from lupus patients displayed an abnormal phenotype characterized by accelerated differentiation, maturation, and secretion of proinflammatory cytokines, suggesting that they are in a preactivated state. Interesting effects of estrogens on the function as well as cytokine secretion by dendritic cells and monocyte / macrophage have been demonstrated. Indeed, Douin-Echinard et al. confirmed that estrogens are required to generate optimal numbers of fully functional dendritic cells *in vitro*, while they induce secretion of IL-6 and IL-12; of note, these effects of E2 were dependent on ERα and not ERβ (115). E2 increased the B cell-stimulating IL-10 production by monocytes(116). Moreover, estrogen can enhance release of IL-6, TNF-a and interleukin-1 (IL-1), from human activated monocytes and / or macrophages, through modulation of CD16 expression (117), although other pathways not involving CD16, whether or not ER-mediated, have previously been recognized (118),(119),(120). At the same line, E2 was found to increase TNF-a, IL-6, and TGF-β secretion by differentiated monocyte / macrophages(121). It should be noted that in regard to TNF-a and IL-1β, the existing literature is at present inconsistent, with E2 either enhancing or inhibiting their secretion by

macrophages / monocytes The divergent results concerning the estrogen effect on the expression of various cytokines would seem to be attributable to differences in the cell type, in concentrations of E2, in type of experiment (*in vivo* or *in vitro*) as well as in duration (shortterm vs longterm) of E2 exposure. In support of the latter, Calippe et al. (112). exhibited the fact that chronic *in vivo* E2 administration promotes the expression of IL-1β, IL-6, IL-12p40 by macrophages in response to TLR4 activation, whilst short-term *in vitro* exposure in E2 decreased the expression of these inflammatory mediators. All the above data strongly suggest that estrogens by acting via their receptors and their crosstalk with other transcription factors in immune cells and organs can modulate immunological parameters and processes that have been shown to be implicated in the pathogenesis of SLE.

6. Effects of estrogen

The actions of steroid hormones can bedivided into two types: those that are delayed in onset and prolonged in duration are called “genomic” effects, and those that are rapid in onset and short in duration are called “non-genomic” effects. The early effects take place within minutes (e.g., changes in vasomotor tone) and are mediated by rapid intracellular signaling pathways, whereas the delayed effects (e.g., remodeling or lipid alterations) require hours to days to occur and require transcriptional events with subsequent modulation of protein expression. Although the rapid and delayed effects of steroids are clearly distinguishable from each other, there are actions that have onset time of minutes and it is not clear as to whether genomic or non-genomic mechanisms apply.

7. Molecular Mechanisms of Estrogen Action

7.1. Nuclear Actions.

Estrogens exert their effects by activating their intracellular receptors, the estrogen receptor alpha (ERα) and beta (ERβ) isoform, encoded by their respective genes ESR1 and ESR2 (122),(123). These receptor proteins belong to the steroid receptor superfamily (124), and possess different sizes.Whereas ERα is comprised of 595 aminoacids, ERβ is comprised of 530 (today known as ERβ1 long). It should be pointed out that the original human ERβ clone encoded a protein of 485 aminoacids (today known as ERβ1 short). The ERβ1 long form is currently regarded as the fulllength wild type ERβ *(125).* Their aminoacid sequences are organized as follows: the ligand binding domain located in the carboxyterminal region of the molecules, necessary for ligand binding; the DNA-binding domain, responsible for binding to specific DNA sequences (the Estrogen Response Elements, EREs); and the transcriptional regulation domain (AF-1), which is highly immunoreactive and is located in the aminoterminal part of the molecules. ERα and ERβ exhibit high homology in their DNA binding domain (96%), low homology (30%) in their AF-1 domain and partial homology (53%) in their ligand binding domain. Various ERα and ERβ isoforms and splicing variants (hERβ1 long, hERβ1 short, hERβ2, hERβ4, hERβ5, hERα-46) have been described (125),(126). ERα and ERβ mediate their effects via regulation of transcription of target genes, directly or indirectly, liganddependently or ligand-independently. Hereinafter, the term ER refers to both ERα and ERβ subtypes. In the absence of hormone, ER is bound to amultiprotein complex, including heat shock proteins, which render the receptor inactive. The ligand binding (endogenous estrogens or exogenous synthetic estrogen analogs) induces a conformational change to the receptor, which results in the release

of bound accessory proteins, and promotes homodimerization of ER subtypes (ERα and ERβ may homodimerize or heterodimerize) and high affinity binding to consensus EREs (AGGTCAnnnTGACCT) (127), located within the regulatory region of target genes (128). The DNA bound receptors interact with many other coactivator proteins and negative coregulators / corepressors, resulting in the stabilization of a transcription preinitiation complex and the remodeling of chromatin and initiation of transcription by the basal transcriptional machinery and RNA polymerase (129),(130),(131). Histone acetyltransferases (HATs) and histone deacetylases (HDACs) are key elements for the chromatin decompaction and initiation, such activities usually possessed by coactivator / corepressor protein complexes (130),(132),(133). ERα and ERβ may regulate gene transcription not only via direct binding to their consensus EREs but also by their interaction with other transcription factors such as the AP-1 (via protein-protein interactions) and modulation of the binding of AP-1 to AP-1 binding sites onto DNA (AP-1 responsive elements), thus regulating AP-1 depending gene transcription(134). ERα and ERβ respond differently to certain ligands in stimulating the ERE or AP-1 pathway(135). For example, tamoxifen is a strong agonist of AP-1 dependent transcription in certain tissues (uterus, endometrium), promoting growth, while it acts as an antiestrogen via the classical EREs, in breast cancer tissue (136). Similarly, estrogen receptor may interact with NF-κB (via proteinprotein interactions), resulting in modulation of the binding of NF-κB to NF-κB binding sites on the DNA (NF-κb response elements), thus regulating NF-κB dependent gene transcription. The estrogen receptor regulates NF-κB mediated gene expression in a cell-type-specific manner and has important implications in inflammatory processes (137),(138). Extracellular signals such as insulin, IGF-1, EGF, and TGF-β, phosphorylate ERα and ERβ (in the AF-1 region) via the mitogen activated protein kinases (MAPKs) in the absence of ligand, resulting in transactivation of the receptors and initiation of ERE-mediated gene expression(139). Phosphorylation is a posttranslational modification of ERs on serine and tyrosine residues. Cellular enzymes such as kinases and phosphatases transfer a phosphate group from ATP onto target proteins or remove it, respectively. Estrogens as well as extracellular signals and regulators of the general cellular phosphorylation state (e.g. protein kinase A, protein kinase C, cyclin dependent kinases) (139), phosphorylate ERs, thus affecting ER functions, positively or negatively, such as transcriptional activity, stability and nucleocytoplasmic shuttling. Estradiol, cell cycle regulators, the enzymes PKA, PKC, extracellular signals such as growth factors, cytokines and neurotransmitters phosphorylate human ERα at serine residues in the aminoterminal A / B domain (serine-104, -106, -118, -154, -167). Phosphorylation sites at serines in DBD, hinge region, and LBD (ser-236, -294, -305) have also been identified. Phosphorylation at ser-118 in human ERα is considered the consensus phosphorylation site for MAPKs and potentiates the interaction with coactivators, while phosphorylation of tyr-537 regulates ERα ligand binding. All together, the ER-mediated signaling cascade, that is, ligand binding, dimerization, DNA binding and interaction with cellular cofactors, seems to be regulated by phosphorylation of ERα (summarized in(140). Studies on ERβ phosphorylation suggest that ser-106 and ser-94 phosphorylation sites at the A / B domain modulate subnuclear mobility of the receptor, the ERβ cellular levels, indicating thus that these phosphorylated serine residues generate signals to the ubiquitin proteasome pathway(141). Phosphorylation at ser-106 and ser-124 also enhance the interaction with the coactivator SRC-1. Phosphorylation at ser-87 has been identified as a regulator of ER-dependent gene expression and growth of breast cancer cells(142). [52]. Collectively, in the presence of diverse physiological signals other than their cognate ligand, the ERs may be activated, this accompanied by an increased phosphorylation state, a pathway which may be responsible for

tissue-specific responses, especially in cases where the concentration of these extracellular signals (e.g., growth factors) is locally increased and estrogens are too low.

8. Extranuclear Actions

8.1. Plasma Membrane ER.

Studies demonstrating that estrogens can cause effects too fast to be based on transcriptional events led to the identification of membrane estrogen receptors responsible for non-genomic responses. The estrogen receptor membrane form exists as a fulllength ER, as an isoform, or as a distinct receptor(143),(144),(145). Membrane forms for both ERα and ERβ have been identified (146). Themembrane localization of ER has been shown to be mediated by the adaptor protein Shc and insulin-like growth factor I receptor(147), while interaction with caveolin-1 and palmitoylation occurring on a specific cysteine (C447) ER residue also plays an important role in this localization(147),(148). Accumulated evidence strongly supports the importance of plasma membrane estrogen receptors in a variety of cells. GPR30, a G protein-coupled receptor (GPCR), binds E2 with high affinity and generates rapid effects(148),(32), including stimulating Ca2+ mobilization from intracellular stores directly or via epidermal growth factor receptor (EGFR) transactivation, c-fos expression, adenyl cyclase, and cAMP mediated signaling and ERK-1/2 in a variety of cell types (2).
Additional signal transduction pathways that are rapidly responsive to E2 and originate from the membrane involve the stimulation/inhibition (being dependent on the ERα or ERβ subtype) of phosphoinositol-3hydroxy kinase (PI3K) and the family of MAP kinases, such as extracellularregulated kinase (ERK), p38β isoform, as well as the c-Jun N-terminal kinase (JNK) (149). Indeed, E2 has been shown to bind the transmembrane G-protein-coupled receptor homolog GPR30 and to activate p44/42 MAPK through transactivation of EGFR (150),(33). Of note, the signaling from the membrane can also extend to other intracellular transcription factors such as AP-1 or NF-kB, resulting in gene repression or activation. The non-genomic actions of estrogens seem to be implicated in important functions such as cell growth, proliferation, differentiation and apoptosis of various cell types (endothelial, bone, neuronal cells, etc.). With regard to the immune system, there are studies confirming the presence of the membrane ERα receptor in peripheral blood mononuclear cells (PBMCs), with estradiol inducing NO release and calcium flux through binding to this receptor. It is of interest that the presence of tamoxifen did not antagonize this effect (151), Moreover, estradiol acting in human monocytes induced the rapid release of NO through membrane ERα and/or ERβ (151). Benten et al. (152). confirmed the existence of membrane ER in T-cells; according to their study, binding of estradiol to membrane ER resulted in release of NO, an effect that was not abolished by the presence of tamoxifen.

8.1.2. Mitochondrial ER.

Accumulating evidence has demonstrated that estrogens exert substantial effects on mitochondrial function, some of these effects being the following: (a) estrogens are potent stabilizers of ATP production during oxidative stress, while under basal conditions they show little effect on mitochondrial ATP production; (b) estrogens prevent Ca++ influx into mitochondria under high excitotoxic stimulation; (c) estrogens protect mitochondria by preventing mitochondrialmembrane potential collapse(153). More importantly, E2—regulates the expression of mtDNAencoded respiratory chain subunits such as cytochrome oxidase

subunits I, II, III in various cell types / tissue (154),(155),(156). Several laboratories have reported the localization of ERα and ERβ in mitochondria in various target cells and tissues by a variety of techniques such as immunohistochemistry, immunocytochemistry, and immunoblots using a wide range of antibodies. A number of studies have demonstrated the presence of ERα in mitochondria of female rat cerebral blood vessels, MCF-7 cells, HepG2 cells, and the 2C12 murine skeletal muscle cell line. Accumulating evidence supports the presence of ERβ in the mitochondria in rabbit uterus and ovary, in MCF-7 cells, in endothelial cells, in primary neurons, primary cardiomyocytes, murine hippocampus cell lines and human heart cells, in human lens epithelial cells, human liver cancer HepG2 cells, osteosarcoma SaOS-2, sperm, and periodontal ligament cells (153),(157). The localization of ERβ in mitochondria has also been verified by proteomics. It should be noted that the wild type human ERβ (known as ERβ1), and not the isoforms ERβ2 to ERβ5, is preferentially localized in the mitochondria (153),(157). It is noteworthy that sequences showing partial similarity to ERE consensus sequence have been detected in the D-Loopregion, the major regulatory region of the mitochondrial genome (158),(159). Several lines of evidence have demonstrated that ERα and ERβ exhibit specific binding to these mtEREs (160), while the mitochondrial ERβ in immortalized human breast epithelial cells (contain ERβ only) has been directly associated with E2-induced expression of mtDNA-encoded respiration chain subunits (COXI, COXII of complex IV and ND1 of complex I) (161). Collectively, the above data support the hypothesis that the mitochondrial genome may be a primary site of action of estrogens (158). It is important to mention that an elevation of the mitochondrial transmembrane potential and ATP depletion has been observed in circulating lymphocytes of patients with SLE (162),(163). Moreover, lupus T cells overexpress genes involved in mitochondrial electron transport (164), whereas a borderline association at nt4917 of the ND2 gene (complex I) and a significant association of the variant at nt9055 in the ATP6 or F0F1-ATPase gene (complex V) have been demonstrated in SLE patients (165). The above data imply that the estrogen-mitochondria crosstalk may be of importance in the pathophysiology of SLE disease.

9. Effects of estrogen on the arterial wall

Actions of steroidal hormones on the arterial wall include alteration or modulation of ion fluxes and of receptors on smooth muscle cells and modulation of endothelium- derived factor production and activity. In this review we focused on the effects of estrogen on components of endothelial and smooth muscle cells. However, it is important to keep in mind that estrogen-induced effects depend on the vascular bed and on the animal species being considered, indicating that there is regional and species heterogeneity in the modulatory influence of estrogen on vasomotor function. To mentionfew examples, it has been reported that 17ßestradiol induces both endothelium-dependent and -independent relaxation in the rat aorta but only endothelium-independent relaxation in the rat mesenteric arteries. NO contributes strongly to the endotheliumdependent relaxation induced by 17ß-estradiol in isolated aortas, whereas in small cerebral arteries both NO and cyclooxygenase (COX) metabolites contribute to estrogeninduced effects. Estrogen treatment increases aortic stiffness and potentiates endothelial vasodilator function in the hindquarters, but not in the carotid vascular bed. Differences in the mechanisms involved in estrogen actions may reflect a differential contribution of mechanisms involved in vascular tone regulation. Furthermore, there is

evidence that ER expression may change with pathological conditions or, inversely, that changes in ER expression may lead to abnormal vascular function (166).

10. Actions of estrogen on endothelial cells

The endothelium plays a major role in vascular tone control by releasing both relaxing and contractile factors and estrogens exert a number of effects on endothelialderived factors, as summarized below. Estrogens have been shown to enhance endothelial-dependent relaxation in arterial rings from different animals and from different vascular beds, including coronary, mesenteric, aorta and cerebral arteries. Studies on humans have demonstrated that estrogen replacement treatment increases coronary flow and decreases both coronary resistance and peripheral vascular tone.

11. Nitric oxide

Earlier reports indicated that basal release of NO is increased in females compared to males (167),(168), and that estrogen administration to ovariectomized rats restores the impaired *ex vivo* basal release of NO. Effects of estradiol were also described in arteries from male animals. Huang et al.(169), observed that 17ß-estradiol restores endothelial NO release in response to shear stress in pressurized gracilis muscle arterioles of male spontaneously hypertensive rats (SHR) by up-regulation of endothelial nitric oxide synthase (NOS). Conversely, it has been reported that endothelium-dependent relaxation elicited by carbachol and histamine was attenuated by estradiol in preparations from intact male rats. Moreover, aortic prostacyclin release was reduced by about 40% after estradiol treatment in tissues from these animals. These results showing that release of NO in arteries from male rats is not affected by estradiol treatment suggest gender specificity for the vascular effects of estrogen. NO production accounts for most of the endothelium-dependent relaxation activity, and there is extensive evidence showing estrogen- induced up-regulation of endothelial NO production. Probable mechanisms involved in estradiol-induced increased NO production include: 1) transcriptional stimulation of NOS gene expression, 2) inhibition of cytokine-induced down-regulation of NOS gene expression, 3) post-translational modification of NOS protein, 4) increased cofactor or L-arginine availability, 5) non-genomic activation of second messengers (e.g., Ca2+, cAMP) and tyrosine kinase, 6) translocation from the membrane to intracellular sites, and 7) modulation of NO degrading systems (e.g., reactive oxygen radical generation and antioxidants). Induction of constitutive (Ca2+-dependent) NOS by estrogen has been demonstrated in a variety of tissues, consistent with the presence of estrogen-response elements in the NOS promoter. In addition to increasing NOS production, estrogen induces rapid enhancement of NOS activity and NO release through nontranscriptional mechanisms and by reducing its Ca2+ dependence(170). Thiseffect seems to be much more intense and functionally relevant than the increase in NOS expression induced by estrogen and is inhibited by the ER antagonist ICI 182,780, indicating that the effect is mediated by ERs(171). In SHR, estrogen deprivation (induced by ovariectomy) decreases NOS activity and expression and NO-derived metabolites(172). Recent studies indicate that estrogen-induced activation of endothelial NOS is driven by activation of the PI3-kinase / Akt pathway resulting from direct interactions between the ER and the regulatory subunit of PI3- kinase (6), and requires MAPK activation(171). Hisamoto et al.(173), observed that 17ßestradiol, but not 17□-estradiol, caused acute activation

of endothelial NOS both in human umbilical vein endothelial cells and in simian virus 40-transformed rat lung vascular endothelial cells. Activation of endothelial NOS involves the activation of Akt and the phosphorylation of endothelial NOS, which is mediated by ER-□□via a non-genomic mechanism. The effects of estrogen on NOS may also be associated with its effects on caveolin-1 expression, which inhibits endothelial NOS catalytic activity. Jayachandran et al.(174). have shown that endothelial NOS protein expression and nitrite / nitrate production by bovine aortic endothelial cells are enhanced by 17ß-estradiol, which also stimulates caveolin-1 transcription and translation through ER-mediated mechanisms. Similar to estrogen, the SERM raloxifene stimulates endothelial NOS mRNA expression (genomic effects) and also triggers rapid activation of NO synthesis by stimulating endothelial NOS (non-genomic effects) via the PI3-kinase pathway ER signaling(175). In femoral veins, raloxifene induces acute relaxation both by NO release and by direct stimulation of vascular smooth muscle cells depending on the ovarian hormonal status of the animal. As we will discuss later, estrogen also prevents NO degradation due to its antioxidant properties, consequently increasing NO availability. The effects of estrogen on NOS activity are suggested to be important in arterial injury. Local delivery of 17ß-estradiol during percutaneous transluminal coronary angioplasty improved endothelial function, enhanced re-endothelialization and endothelial NOS expression and decreased neointima formation. Recently, Tolbert et al.(176), have shown that the vasoprotective effects of estrogen after ligation vascular injury are partially reduced in inducible NOS knockout mice, suggesting that estrogen also modulates inducible NOS expression and plays a role in neointima formation.

12. Inflammatory pathologies regulated by estrogen

Abnormal regulation of the immune system could lead to various complications in female reproduction. Various autoimmune and inflammatory disorders have been reported (177),(1),(178, 179). Estrogens have been implicated directly in diseases like arthritis, osteoporosis, systemic lupus erythematosus, multiple sclerosis, preeclampsia, complications in fertility, pregnancy loss, post-term labor, labor complications, cancers of breast and reproductive tract. Estrogens also play a vital role in the pathophysiology of female reproduction mediated by leukocytes. There are ample evidences to indicate that aberrant inflammatory pathways are directly or indirectly regulated by estrogens, contributing to the cause of various diseases.

13. Estrogen receptors in leukocytes

Leukocytes play a key role in several physiologically important processes like immunity, inflammation, extracellular matrix remodeling, wound healing, cardiovascular disorders, autoimmune diseases, menstruation, embryo implantation, cervical ripening, labor etc. They are involved in various functions during normal as well as pathological conditions. Estrogens act on leukocytes and influence their number and function (180).
In recent years, several investigations have focused on the action of estrogens in the immune Update on Mechanisms of Hormone Action – Focus on Metabolism, Growth 338 and Reproduction system and inflammation. Clinical, epidemiological and immunological studies have shown that women are more prone to autoimmune disorders in comparison to men. Studies have shown that the incidence of cardiovascular disease is higher in men than in women and the incidence in women increases towards the level of men after menopause. There

is clear sex bias in the disease presentation. Estrogens have been suggested to be responsible for these differences (177),(181),(182). These diseases are often associated with leukocyte infiltration and immune dysfunction. It has been hypothesized that estrogens alter the course of these disorders by modulating leukocyte function in various tissues. Although the exact mechanism by which estrogens modulates the immune cell function is not completely understood, these observations clearly show that leukocytes are estrogen targets.

13.1. Neutrophils

Neutrophils are the most abundant type of leukocytes and form an essential part of the immune system. Klebanoff demonstrated that estrogens specifically bind to neutrophils using ligand binding experiments(183). It was further shown that estrogens influence the neutrophil count and women have a higher neutrophil count than men(184). In women, the neutrophil number varies during the menstrual cycle(185),(186). Higher levels of neutrophil counts correlate to the elevated levels of estradiol in peripheral blood(187). Recent studies showed that ERs are present in neutrophils and execute various direct or indirect functions. It was shown that polymorphonuclear cells express both ERα and ERβ and their various splice variants(188),(189). Molero et al, demonstrated that estradiol up-regulated both ERα and ERβ in women but only ERα in men (188). The functional signaling of ERs in neutrophils was further established by the induction of nNOS by estradiol(190). Further, estradiol and ER specific agonists regulated physiologically relevant genes in polymorphonuclear cells in rats(189). Recently, we have identified the presence of GPER in terminally differentiated neutrophil like HL-60 cells. The GPER agonist G1 could stimulate a transcriptional response indicating that GPER is functionally active in these cells (Blesson and Sahlin, unpublished). Neutrophils have a very short life span and they stay in circulation for 6 to 18 hours before undergoing apoptosis. Estradiol along with progesterone increases neutrophil survival by delaying apoptosis via decreasing the activities of caspases 3 and 9(191). Estrogens may also have a vital role in the regulation of genes that are associated with the immune and inflammatory response, like chemokines and cytokines. These genes are responsible for neutrophil recruitment and activation during normal as well as pathological conditions(178),(179).

13.2. Lymphocytes

Lymphocytes express nuclear as well as membrane estrogen receptors. Studies on human peripheral blood lymphocytes showed the presence of ERα and ERβ in various lymphocyte subsets including natural killer (NK) cells (192). A smaller variant of ERα and ERβ 46 appears to be the most abundant isoform of ERs in lymphocytes. This variant was localized to the cell surface and mediates estrogen induced proliferation of T lymphocytes and NK cells but not B lymphocytes Estrogen Receptors in Leukocytes - Possible Impact on Inflammatory Processes in the Female Reproductive System 339 2010). ER is expressed predominantly in secondary lymphoid tissues and plays an important role in the peripheral immune system(193). Both ERα and ERβ are expressed in the NK cells of mice and humans (192). In mice, estrogens act via ER to suppress NK cell activity by altering their ability to lyse target cells Estradiol induces the proliferation of splenic NK cells and suppresses the cytotoxicity of these cells (194). However, *in vitro* studies on murine NK cells showed that estradiol reduces NK cell proliferative capacity and reduces cytotoxicity by influencing cytokine expressions(195). In humans, the number of

NK cells was significantly altered during the different phases of menstrual cycle. The NK cell population in the phase when the estrogen level is high was twice that in other phases indicating a positive effect on its number (196). The ER1 variant could be localized to uterine NK (uNK) cells (197). Hence, estrogens could act directly on uNK cells via the ER1 receptor.

14. Conclusion

Estrogen is active both in vascular smooth muscle and endothelial cells and may exert its cardiovascular protective actions by a direct effect on the vessel wall. Clinical and animal studies have demonstrated the beneficial effects of estrogen on the vascular system. Estrogens act through ERs and regulate various aspects of the immune system directly or indirectly acting through various downstream mediators. ERs have been found on diverse types of leukocytes. Estrogens act directly via its different receptors and regulate various inflammatory functions mediated through different types of leukocytes. Estrogens are also able to regulate the number, migration and function of leukocytes involving complex mechanisms. Considering the recent findings of the function of estrogens in various aspects of immune regulation and inflammation, it is difficult to consider estrogens just as a 'female reproductive hormone' anymore. The role of estrogens in various inflammatory processes and its significance is well accepted. GPER-selective agents that mimic the beneficial effects of 17β-estradiol without its associated feminizing or other adverse effects could represent an important new family of drugs.
In addition, GPER-specific antagonists could be developed as important additions to the armamentarium of drugs used to treat estrogen-sensitive cancers and other diseases in which estrogen signaling is important. In this regard, the potential contribution of GPER-mediated signaling to the effects of existing clinically approved drugs, such as tamoxifen and fulvestrant, must be considered. GPER-mediated effects should also be taken into account in the future development of SERMs and SERDs. Possible correlations of ER gene polymorphisms and of quantitative and qualitative changes in the receptor proteins to cytokine production and to disease aetiopathogenesis have also been reported. Recent evidence indicates a role of estrogens in mitochondrial function in immune cells along with cytokine regulation, while the existence of mitochondrial ER in human cells has been associated with stimulation of mitochondrial encoded enzymes. The above data, together with the recent findings that SLE patients are characterized by mitochondrial dysfunction, suggest that novel pathways of the estrogen- ER complex in mitochondria in immune cells may play a key role in SLE. Therefore, Insights into the function and regulation of ERs in leukocytes could open up new possibilities for treatments for various diseases involving inflammation. Furthermore, the beneficial clinical effects of estrogen need to be confirmed in large and multicenter randomized clinical trials

15. Reference

1. Deroo BJ, Korach KS. Estrogen receptors and human disease. The Journal of clinical investigation. 2006;116(3):561-70.
2. Prossnitz ER, Arterburn JB, Smith HO, Oprea TI, Sklar LA, Hathaway HJ. Estrogen signaling through the transmembrane G protein-coupled receptor GPR30. Annu Rev Physiol. 2008;70:165-90.

3. Edwards DP. Regulation of signal transduction pathways by estrogen and progesterone. Annu Rev Physiol. 2005;67:335-76.
4. Lóránd T, Vigh E, Garai J. Hormonal action of plant derived and anthropogenic non-steroidal estrogenic compounds: phytoestrogens and xenoestrogens. Current medicinal chemistry. 2010;17(30):3542-74.
5. Prossnitz ER, Arterburn JB, Sklar LA. GPR30: AG protein-coupled receptor for estrogen. Molecular and cellular endocrinology. 2007;265:138-42.
6. Stygar D, Westlund P, Eriksson H, Sahlin L. Identification of wild type and variants of oestrogen receptors in polymorphonuclear and mononuclear leucocytes. Clinical endocrinology. 2006;64(1):74-81.
7. Talwar G, Segal S, Evans A, Davidson O. The binding of estradiol in the uterus: A mechanism for derepression of RNA synthesis. Proceedings of the National Academy of Sciences. 1964;52(4):1059-66.
8. Soloff MS, Szego CM. Purification of estradiol receptor from rat uterus and blockade of its estrogen-binding function by specific antibody. Biochemical and biophysical research communications. 1969;34(1):141-7.
9. Kuiper G, Enmark E, Pelto-Huikko M, Nilsson S, Gustafsson J-A. Cloning of a novel receptor expressed in rat prostate and ovary. Proceedings of the National Academy of Sciences. 1996;93(12):5925-30.
10. Carroll JS, Brown M. Estrogen receptor target gene: an evolving concept. Molecular Endocrinology. 2006;20(8):1707-14.
11. Hewitt SC, Korach KS. Oestrogen receptor knockout mice: roles for oestrogen receptors alpha and beta in reproductive tissues. Reproduction. 2003;125(2):143-9.
12. Hammes SR, Levin ER. Extranuclear steroid receptors: nature and actions. Endocrine reviews. 2007;28(7):726-41.
13. Pietras RJ, Szego CM. Endometrial cell calcium and oestrogen action. 1975.
14. Pietras RJ, Szego CM. Specific binding sites for oestrogen at the outer surfaces of isolated endometrial cells. 1977.
15. Szego CM, Davis JS. Adenosine 3', 5'-monophosphate in rat uterus: acute elevation by estrogen. Proceedings of the National Academy of Sciences. 1967;58(4):1711-8.
16. Wehling M. Specific, nongenomic actions of steroid hormones. Annual review of physiology. 1997;59(1):365-93.
17. Filardo E, Quinn J, Bland K, Frackelton Jr A. Jr 2000. Estrogen-induced activation of Erk-1 and Erk-2 requires the G protein-coupled receptor homolog, GPR30, and occurs via trans-activation of the epidermal growth factor receptor through release of HB-EGF Mol Endocrinol.14:1649-60.
18. Carmeci C, Thompson DA, Ring HZ, Francke U, Weigel RJ. Identification of a gene (GPR30) with homology to the G-protein-coupled receptor superfamily associated with estrogen receptor expression in breast cancer. Genomics. 1997;45(3):607-17.
19. Feng Y, Gregor P. Cloning of a novel member of the G protein-coupled receptor family related to peptide receptors. Biochemical and biophysical research communications. 1997;231(3):651-4.
20. Kvingedal AM, Smeland EB. A novel putative G-protein-coupled receptor expressed in lung, heart and lymphoid tissue. FEBS letters. 1997;407(1):59-62.
21. O'Dowd BF, Nguyen T, Marchese A, Cheng R, Lynch KR, Heng HH, et al. Discovery of three novel G-protein-coupled receptor genes. Genomics. 1998;47(2):310-3.

22. Owman C, Blay P, Nilsson C, Lolait SJ. Cloning of human cDNA encoding a novel heptahelix receptor expressed in Burkitt's lymphoma and widely distributed in brain and peripheral tissues. Biochemical and biophysical research communications. 1996;228(2):285-92.
23. Takada Y, Kato C, Kondo S, Korenaga R, Ando J. Cloning of cDNAs encoding G protein-coupled receptor expressed in human endothelial cells exposed to fluid shear stress. Biochemical and biophysical research communications. 1997;240(3):737-41.
24. Filardo EJ, Quinn JA, Frackelton Jr AR, Bland KI. Estrogen action via the G protein-coupled receptor, GPR30: stimulation of adenylyl cyclase and cAMP-mediated attenuation of the epidermal growth factor receptor-to-MAPK signaling axis. Molecular endocrinology. 2002;16(1):70-84.
25. Kanda N, Watanabe S. 17β-estradiol inhibits oxidative stress-induced apoptosis in keratinocytes by promoting Bcl-2 expression. Journal of Investigative Dermatology. 2003;121(6):1500-9.
26. Kanda N, Watanabe S. 17β-estradiol enhances the production of nerve growth factor in THP-1-derived macrophages or peripheral blood monocyte-derived macrophages. Journal of investigative dermatology. 2003;121(4):771-80.
27. Kanda N, Watanabe S. 17β-estradiol stimulates the growth of human keratinocytes by inducing cyclin D2 expression. Journal of Investigative Dermatology. 2004;123(2):319-28.
28. Maggiolini M, Vivacqua A, Fasanella G, Recchia AG, Sisci D, Pezzi V, et al. The G protein-coupled receptor GPR30 mediates c-fos up-regulation by 17β-estradiol and phytoestrogens in breast cancer cells. Journal of Biological Chemistry. 2004;279(26):27008-16.
29. Ahola TM, Alkio N, Manninen T, Ylikomi T. Progestin and G protein-coupled receptor 30 inhibit mitogen-activated protein kinase activity in MCF-7 breast cancer cells. Endocrinology. 2002;143(12):4620-6.
30. Ahola TM, Manninen T, Alkio N, Ylikomi T. G protein-coupled receptor 30 is critical for a progestin-induced growth inhibition in MCF-7 breast cancer cells. Endocrinology. 2002;143(9):3376-84.
31. Ahola TM, Purmonen S, Pennanen P, Zhuang YH, Tuohimaa P, Ylikomi T. Progestin upregulates G-protein-coupled receptor 30 in breast cancer cells. European Journal of Biochemistry. 2002;269(10):2485-90.
32. Thomas P, Pang Y, Filardo E, Dong J. Identity of an estrogen membrane receptor coupled to a G protein in human breast cancer cells. Endocrinology. 2005;146(2):624-32.
33. Revankar CM, Cimino DF, Sklar LA, Arterburn JB, Prossnitz ER. A transmembrane intracellular estrogen receptor mediates rapid cell signaling. Science. 2005;307(5715):1625-30.
34. Olde B, Leeb-Lundberg LF. GPR30/GPER1: searching for a role in estrogen physiology. Trends in Endocrinology & Metabolism. 2009;20(8):409-16.
35. Boon WC, Chow JD, Simpson ER. The multiple roles of estrogens and the enzyme aromatase. Progress in brain research. 2010;181:209-32.
36. Lappano R, Rosano C, De Marco P, De Francesco EM, Pezzi V, Maggiolini M. Estriol acts as a GPR30 antagonist in estrogen receptor-negative breast cancer cells. Molecular and cellular endocrinology. 2010;320(1):162-70.
37. Pasqualini J, Gelly C, Nguyen B-L, Vella C. Importance of estrogen sulfates in breast cancer. Journal of steroid biochemistry. 1989;34(1-6):155-63.
38. Geisler J. Breast cancer tissue estrogens and their manipulation with aromatase inhibitors and inactivators. The Journal of steroid biochemistry and molecular biology. 2003;86(3):245-53.

39. Diczfalusy E, Mancuso S. Oestrogen metabolism in pregnancy. Foetus and placenta. 1969:191-248.
40. Muller R, Johnston T, Traish A, Wotiz H. Studies on the mechanism of estradiol uptake by rat uterine cells and on estradiol binding to uterine plasma membranes. Steroid Hormone Receptor Systems: Springer; 1979. p. 401-21.
41. Ososki AL, Kennelly EJ. Phytoestrogens: a review of the present state of research. Phytotherapy Research. 2003;17(8):845-69.
42. Starek A. Estrogens and organochlorine xenoestrogens and breast cancer risk. International Journal of Occupational Medicine and Environmental Health. 2003;16(2):113-24.
43. Singleton DW, Khan SA. Xenoestrogen exposure and mechanisms of endocrine disruption. Front Biosci. 2003;8:s110-s8.
44. Morton MS, Arisaka O, Miyake N, Morgan LD, Evans BA. Phytoestrogen concentrations in serum from Japanese men and women over forty years of age. The Journal of Nutrition. 2002;132(10):3168-71.
45. Thomas P, Dong J. Binding and activation of the seven-transmembrane estrogen receptor GPR30 by environmental estrogens: a potential novel mechanism of endocrine disruption. The Journal of steroid biochemistry and molecular biology. 2006;102(1):175-9.
46. Chevalier N, Bouskine A, Fenichel P. Bisphenol A promotes testicular seminoma cell proliferation through GPER/GPR30. International Journal of Cancer. 2012;130(1):241-2.
47. Albanito L, Lappano R, Madeo A, Chimento A, Prossnitz ER, Cappello AR, et al. G-Protein–Coupled Receptor 30 and Estrogen Receptor-α Are Involved in the Proliferative Effects Induced by Atrazine in Ovarian Cancer Cells-RETRACTED. Environmental health perspectives. 2008;116(12):1648-55.
48. Rowlands DJ, Chapple S, Siow RC, Mann GE. Equol-stimulated mitochondrial reactive oxygen species activate endothelial nitric oxide synthase and redox signaling in endothelial cells roles for F-Actin and GPR30. Hypertension. 2011;57(4):833-40.
49. Jordan VC. SERMs: meeting the promise of multifunctional medicines. Journal of the National Cancer Institute. 2007;99(5):350-6.
50. Orlando L, Schiavone P, Fedele P, Calvani N, Nacci A, Rizzo P, et al. Molecularly targeted endocrine therapies for breast cancer. Cancer treatment reviews. 2010;36:S67-S71.
51. Fitts JM, Klein RM, Powers CA. Tamoxifen regulation of bone growth and endocrine function in the ovariectomized rat: discrimination of responses involving estrogen receptor α/estrogen receptor β, G protein-coupled estrogen receptor, or estrogen-related receptor γ using fulvestrant (ICI 182780). Journal of Pharmacology and Experimental Therapeutics. 2011;338(1):246-54.
52. Albanito L, Madeo A, Lappano R, Vivacqua A, Rago V, Carpino A, et al. G protein–coupled receptor 30 (GPR30) mediates gene expression changes and growth response to 17β-estradiol and selective GPR30 ligand G-1 in ovarian cancer cells. Cancer research. 2007;67(4):1859-66.
53. Dennis MK, Field AS, Burai R, Ramesh C, Petrie WK, Bologa CG, et al. Identification of a GPER/GPR30 antagonist with improved estrogen receptor counterselectivity. The Journal of steroid biochemistry and molecular biology. 2011;127(3):358-66.
54. Blasko E, Haskell CA, Leung S, Gualtieri G, Halks-Miller M, Mahmoudi M, et al. Beneficial role of the GPR30 agonist G-1 in an animal model of multiple sclerosis. Journal of neuroimmunology. 2009;214(1):67-77.
55. Dennis MK, Burai R, Ramesh C, Petrie WK, Alcon SN, Nayak TK, et al. In vivo effects of a GPR30 antagonist. Nature chemical biology. 2009;5(6):421-7.

56. Jenei-Lanzl Z, Straub RH, Dienstknecht T, Huber M, Hager M, Grässel S, et al. Estradiol inhibits chondrogenic differentiation of mesenchymal stem cells via nonclassic signaling. Arthritis & Rheumatism. 2010;62(4):1088-96.
57. Lindsey SH, Carver KA, Prossnitz ER, Chappell MC. Vasodilation in response to the GPR30 agonist G-1 is not different from estradiol in the mRen2. Lewis female rat. Journal of cardiovascular pharmacology. 2011;57(5):598.
58. Peyton C, Thomas P. Involvement of epidermal growth factor receptor signaling in estrogen inhibition of oocyte maturation mediated through the G protein-coupled estrogen receptor (Gper) in zebrafish (Danio rerio). Biology of reproduction. 2011;85(1):42-50.
59. Gingerich S, Kim G, Chalmers J, Koletar M, Wang X, Wang Y, et al. Estrogen receptor alpha and G-protein coupled receptor 30 mediate the neuroprotective effects of 17β-estradiol in novel murine hippocampal cell models. Neuroscience. 2010;170(1):54-66.
60. Nayak TK, Dennis MK, Ramesh C, Burai R, Atcher RW, Sklar LA, et al. Influence of charge on cell permeability and tumor imaging of GPR30-targeted 111in-labeled nonsteroidal imaging agents. ACS chemical biology. 2010;5(7):681-90.
61. Ramesh C, Nayak TK, Burai R, Dennis MK, Hathaway HJ, Sklar LA, et al. Synthesis and characterization of iodinated tetrahydroquinolines targeting the G protein-coupled estrogen receptor GPR30. Journal of medicinal chemistry. 2009;53(3):1004-14.
62. Gruber CJ, Tschugguel W, Schneeberger C, Huber JC. Production and actions of estrogens. New England Journal of Medicine. 2002;346(5):340-52.
63. Nelson LR, Bulun SE. Estrogen production and action. Journal of the American Academy of Dermatology. 2001;45(3):S116-S24.
64. Speroff L, Fritz MA. Clinical gynecologic endocrinology and infertility: lippincott Williams & wilkins; 2005.
65. Park SH, Kim PN, Kim KW, Lee SW, Yoon SE, Park SW, et al. Macrovesicular Hepatic Steatosis in Living Liver Donors: Use of CT for Quantitative and Qualitative Assessment 1. Radiology. 2006;239(1):105-12.
66. Tsokos GC, Kammer GM. Molecular aberrations in human systemic lupus erythematosus. Molecular medicine today. 2000;6(11):418-24.
67. Pernis AB. Estrogen and CD4+ T cells. Current opinion in rheumatology. 2007;19(5):414-20.
68. Mevorach D, Perrot S, Buchanan NM, Khamashta M, Laoussadi S, Hughes GR, et al. Appearance of systemic lupus erythematosus after thymectomy: four case reports and review of the literature. Lupus. 1995;4(1):33-7.
69. Gutierrez-Ramos JC, Andreu JL, Revilla Y, Viñuela E, Martinez C. Recovery from autoimmunity of MRL/lpr mice after infection with an interleukin-2/vaccinia recombinant virus. Nature. 1990;346(6281):271-4.
70. Stimson W, Hunter I. Oestrogen-induced immunoregulation mediated through the thymus. Journal of clinical & laboratory immunology. 1980;4(1):27.
71. Ryan MR, Shepherd R, Leavey JK, Gao Y, Grassi F, Schnell FJ, et al. An IL-7-dependent rebound in thymic T cell output contributes to the bone loss induced by estrogen deficiency. Proceedings of the National Academy of Sciences of the United States of America. 2005;102(46):16735-40.
72. Staples JE, Gasiewicz TA, Fiore NC, Lubahn DB, Korach KS, Silverstone AE. Estrogen receptor α is necessary in thymic development and estradiol-induced thymic alterations. The Journal of Immunology. 1999;163(8):4168-74.

73. Cohen J, Danel L, Cordier G, Saez S, Revillard J. Sex steroid receptors in peripheral T cells: absence of androgen receptors and restriction of estrogen receptors to OKT8-positive cells. The Journal of Immunology. 1983;131(6):2767-71.
74. Suenaga R, Evans M, Mitamura K, Rider V, Abdou N. Peripheral blood T cells and monocytes and B cell lines derived from patients with lupus express estrogen receptor transcripts similar to those of normal cells. The Journal of rheumatology. 1998;25(7):1305-12.
75. Maret A, Coudert JD, Garidou L, Foucras G, Gourdy P, Krust A, et al. Estradiol enhances primary antigen-specific CD4 T cell responses and Th1 development in vivo. Essential role of estrogen receptor α expression in hematopoietic cells. European journal of immunology. 2003;33(2):512-21.
76. Bao M, Yang Y, Jun H-S, Yoon J-W. Molecular mechanisms for gender differences in susceptibility to T cell-mediated autoimmune diabetes in nonobese diabetic mice. The Journal of Immunology. 2002;168(10):5369-75.
77. Entrican G. Immune regulation during pregnancy and host–pathogen interactions in infectious abortion. Journal of comparative pathology. 2002;126(2):79-94.
78. Krishnan L, Guilbert LJ, Russell AS, Wegmann TG, Mosmann TR, Belosevic M. Pregnancy impairs resistance of C57BL/6 mice to Leishmania major infection and causes decreased antigen-specific IFN-gamma response and increased production of T helper 2 cytokines. The Journal of Immunology. 1996;156(2):644-52.
79. Salem ML. Estrogen, a double-edged sword: modulation of TH1-and TH2-mediated inflammations by differential regulation of TH1/TH2 cytokine production. Current Drug Targets-Inflammation & Allergy. 2004;3(1):97-104.
80. Gilmore W, Weiner LP, Correale J. Effect of estradiol on cytokine secretion by proteolipid protein-specific T cell clones isolated from multiple sclerosis patients and normal control subjects. The Journal of Immunology. 1997;158(1):446-51.
81. Correale J, Arias M, Gilmore W. Steroid hormone regulation of cytokine secretion by proteolipid protein-specific CD4+ T cell clones isolated from multiple sclerosis patients and normal control subjects. The Journal of Immunology. 1998;161(7):3365-74.
82. Zang YC, Halder JB, Hong J, Rivera VM, Zhang JZ. Regulatory effects of estriol on T cell migration and cytokine profile: inhibition of transcription factor NF-κB. Journal of neuroimmunology. 2002;124(1):106-14.
83. Lambert KC, Curran EM, Judy BM, Milligan GN, Lubahn DB, Estes DM. Estrogen receptor α (ERα) deficiency in macrophages results in increased stimulation of CD4+ T cells while 17β-estradiol acts through ERα to increase IL-4 and GATA-3 expression in CD4+ T cells independent of antigen presentation. The Journal of Immunology. 2005;175(9):5716-23.
84. Kamada M, Irahara M, Maegawa M, Ohmoto Y, Murata K, Yasui T, et al. Transient increase in the levels of T-helper 1 cytokines in postmenopausal women and the effects of hormone replacement therapy. Gynecologic and obstetric investigation. 2001;52(2):82-8.
85. Kumru S, Godekmerdan A, Yılmaz B. Immune effects of surgical menopause and estrogen replacement therapy in peri-menopausal women. Journal of reproductive immunology. 2004;63(1):31-8.
86. McMurray RW, Ndebele K, Hardy KJ, Jenkins JK. 17-β-estradiol suppresses IL-2 and IL-2 receptor. Cytokine. 2001;14(6):324-33.
87. Xia X, Zhang S, Yu Y, Zhao N, Liu R, Liu K, et al. Effects of estrogen replacement therapy on estrogen receptor expression and immunoregulatory cytokine secretion in surgically induced menopausal women. Journal of reproductive immunology. 2009;81(1):89-96.

88. Segal R, Dayan M, Zinger H, Habut B, Shearer GM, Mozes E. The effect of IL-12 on clinical and laboratory aspects of experimental SLE in young and aging mice. Experimental gerontology. 2003;38(6):661-8.
89. Polan ML, Daniele A, Kuo A. Gonadal steroids modulate human monocyte interleukin-1 (IL-1) activity. Fertility and sterility. 1988;49(6):964-8.
90. Steinman L. A brief history of TH17, the first major revision in the TH1/TH2 hypothesis of T cell–mediated tissue damage. Nature medicine. 2007;13(1):139-45.
91. Nalbandian A, Crispin J, Tsokos G. Interleukin-17 and systemic lupus erythematosus: current concepts. Clinical & Experimental Immunology. 2009;157(2):209-15.
92. Wang C, Dehghani B, Li Y, Kaler LJ, Vandenbark AA, Offner H. Oestrogen modulates experimental autoimmune encephalomyelitis and interleukin-17 production via programmed death 1. Immunology. 2009;126(3):329-35.
93. Rogers A, Eastell R. The effect of 17β-estradiol on production of cytokines in cultures of peripheral blood. Bone. 2001;29(1):30-4.
94. Rachon D, Mysliwska J, Suchecka-Rachon K, Wieckiewicz J, Mysliwski A. Effects of oestrogen deprivation on interleukin-6 production by peripheral blood mononuclear cells of postmenopausal women. Journal of Endocrinology. 2002;172(2):387-95.
95. Kube D, Platzer C, Von Knethen A, Straub H, Bohlen H, Hafner M, et al. Isolation of the human interleukin 10 promoter. Characterization of the promoter activity in Burkitt's lymphoma cell lines. cytokine. 1995;7(1):1-7.
96. Fox HS, Bond BL, Parslow TG. Estrogen regulates the IFN-gamma promoter. The Journal of Immunology. 1991;146(12):4362-7.
97. Baeuerle PA, Henkel T. Function and activation of NF-kappaB in the immune system. Annual review of immunology. 1994;12(1):141-79.
98. Liu J, Beller DI. Distinct pathways for NF-κB regulation are associated with aberrant macrophage IL-12 production in lupus-and diabetes-prone mouse strains. The Journal of Immunology. 2003;170(9):4489-96.
99. Katsiari CG, Tsokos GC. Transcriptional repression of interleukin-2 in human systemic lupus erythematosus. Autoimmunity reviews. 2006;5(2):118-21.
100. Tsokos GC, Wong HK, Enyedy EJ, Nambiar MP. Immune cell signaling in lupus. Current opinion in rheumatology. 2000;12(5):355-63.
101. Rao A. NF-ATp: a transcription factor required for the co-ordinate induction of several cytokine genes. Immunology today. 1994;15(6):274-81.
102. Schwartz RH. Costimulation of T Lymphocytes: Minireview The Role of 0D28, CTLA-4, and B7/BB1 in Interleukin-2 Production and Immunotherapy. Cell. 1992;71:1065-8.
103. Wong HK, Kammer GM, Dennis G, Tsokos GC. Abnormal NF-κB activity in T lymphocytes from patients with systemic lupus erythematosus is associated with decreased p65-RelA protein expression. The Journal of Immunology. 1999;163(3):1682-9.
104. Whiteside ST, Epinat JC, Rice NR, Israël A. I kappa B epsilon, a novel member of the IκB family, controls RelA and cRel NF-κB activity. The EMBO journal. 1997;16(6):1413-26.
105. Herndon TM, Juang Y-T, Solomou EE, Rothwell SW, Gourley MF, Tsokos GC. Direct transfer of p65 into T lymphocytes from systemic lupus erythematosus patients leads to increased levels of interleukin-2 promoter activity. Clinical Immunology. 2002;103(2):145-53.
106. Oikonomidou O, Vlachoyiannopoulos PG, Kominakis A, Kalofoutis A, Moutsopoulos HM, Moutsatsou P. Glucocorticoid receptor, nuclear factor κB, activator protein-1 and C-jun N-terminal kinase in systemic lupus erythematosus patients. Neuroimmunomodulation. 2007;13(4):194-204.

107. Wong H, Kammer G, Mishra N, Dennis G, Tsokos G, editors. Activator protein-1 (AP-1) regulation in lymphocytes from patients with systemic lupus erythematosus. Arthritis and Rheumatism; 1999: LIPPINCOTT WILLIAMS & WILKINS 530 WALNUT ST, PHILADELPHIA, PA 19106-3621 USA.
108. Solomou EE, Juang Y-T, Gourley MF, Kammer GM, Tsokos GC. Molecular basis of deficient IL-2 production in T cells from patients with systemic lupus erythematosus. The Journal of Immunology. 2001;166(6):4216-22.
109. Kyttaris VC, Juang Y-T, Tenbrock K, Weinstein A, Tsokos GC. Cyclic adenosine 5′-monophosphate response element modulator is responsible for the decreased expression of c-fos and activator protein-1 binding in T cells from patients with systemic lupus erythematosus. The Journal of Immunology. 2004;173(5):3557-63.
110. Dajee M, Ehrhardt R, Hofland H, McEvoy L, Muchamuel T, Schryver B. Delivery of polynucleotides. Google Patents; 2005.
111. Hirano S, Furutama D, Hanafusa T. Physiologically high concentrations of 17β-estradiol enhance NF-κB activity in human T cells. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. 2007;292(4):R1465-R71.
112. Calippe B, Douin-Echinard V, Laffargue M, Laurell H, Rana-Poussine V, Pipy B, et al. Chronic estradiol administration in vivo promotes the proinflammatory response of macrophages to TLR4 activation: involvement of the phosphatidylinositol 3-kinase pathway. The Journal of Immunology. 2008;180(12):7980-8.
113. Ding D, Mehta H, McCune WJ, Kaplan MJ. Aberrant phenotype and function of myeloid dendritic cells in systemic lupus erythematosus. The Journal of Immunology. 2006;177(9):5878-89.
114. Decker P, Kötter I, Klein R, Berner B, Rammensee H-G. Monocyte-derived dendritic cells over-express CD86 in patients with systemic lupus erythematosus. Rheumatology. 2006;45(9):1087-95.
115. Douin-Echinard V, Laffont S, Seillet C, Delpy L, Krust A, Chambon P, et al. Estrogen receptor α, but not β, is required for optimal dendritic cell differentiation and CD40-induced cytokine production. The Journal of Immunology. 2008;180(6):3661-9.
116. Kanda N, Tsuchida T, Tamaki K. Estrogen enhancement of anti–double-stranded DNA antibody and immunoglobulin G production in peripheral blood mononuclear cells from patients with systemic lupus erythematosus. Arthritis & Rheumatism. 1999;42(2):328-37.
117. Kramer P, Kramer S, Guan G. 17β-estradiol regulates cytokine release through modulation of CD16 expression in monocytes and monocyte-derived macrophages. Arthritis & Rheumatism. 2004;50(6):1967-75.
118. Pottratz ST, Bellido T, Mocharla H, Crabb D, Manolagas SC. 17 beta-Estradiol inhibits expression of human interleukin-6 promoter-reporter constructs by a receptor-dependent mechanism. Journal of Clinical Investigation. 1994;93(3):944.
119. Srivastava S, Weitzmann MN, Cenci S, Ross FP, Adler S, Pacifici R. Estrogen decreases TNF gene expression by blocking JNK activity and the resulting production of c-Jun and JunD. The Journal of clinical investigation. 1999;104(4):503-13.
120. Krieg SA, Krieg AJ, Shapiro DJ. A unique downstream estrogen responsive unit mediates estrogen induction of proteinase inhibitor-9, a cellular inhibitor of IL-1β-converting enzyme (caspase 1). Molecular Endocrinology. 2001;15(11):1971-82.
121. Cutolo M, Montagna P, Brizzolara R, Sulli A, Seriolo B, Villaggio B, et al. Sex hormones modulate the effects of Leflunomide on cytokine production by cultures of differentiated

monocyte/macrophages and synovial macrophages from rheumatoid arthritis patients. Journal of autoimmunity. 2009;32(3):254-60.
122. Greene GL, Gilna P, Waterfield M, Baker A, Hort Y, Shine J. Sequence and expression of human estrogen receptor complementary DNA. Science. 1986;231(4742):1150-4.
123. Mosselman S, Polman J, Dijkema R. ERβ: identification and characterization of a novel human estrogen receptor. FEBS letters. 1996;392(1):49-53.
124. Curtis SH, Korach KS. Steroid receptor knockout models: phenotypes and responses illustrate interactions between receptor signaling pathways in vivo. Advances in pharmacology. 1999;47:357-80.
125. Matthews J, Gustafsson J-Å. Estrogen signaling: a subtle balance between ERα and ERβ. Molecular interventions. 2003;3(5):281.
126. Moore JT, McKee DD, Slentz-Kesler K, Moore LB, Jones SA, Horne EL, et al. Cloning and characterization of human estrogen receptor β isoforms. Biochemical and biophysical research communications. 1998;247(1):75-8.
127. Mason CE, Shu F-J, Wang C, Session RM, Kallen RG, Sidell N, et al. Location analysis for the estrogen receptor-α reveals binding to diverse ERE sequences and widespread binding within repetitive DNA elements. Nucleic acids research. 2010;38(7):2355-68.
128. Beato M, Herrlich P, Schütz G. Steroid hormone receptors: many actors in search of a plot. Cell. 1995;83(6):851-7.
129. Horwitz K, Jackson T, Bain D, Richer J, Takimoto G, Tung L. Nuclear receptor coactivators and corepressors. Molecular Endocrinology. 1996;10(10):1167-77.
130. Lee KC, Kraus WL. Nuclear receptors, coactivators and chromatin: new approaches, new insights. Trends in Endocrinology & Metabolism. 2001;12(5):191-7.
131. Freedman LP. Increasing the complexity of coactivation in nuclear receptor signaling. Cell. 1999;97(1):5-8.
132. Wolffe AP. Chromatin remodeling regulated by steroid and nuclear receptors. Cell research. 1997;7(2):127-42.
133. Wolffe AP, Hayes JJ. Chromatin disruption and modification. Nucleic acids research. 1999;27(3):711-20.
134. Cerillo G, Rees A, Manchanda N, Reilly C, Brogan I, White A, et al. The oestrogen receptor regulates NFκB and AP-1 activity in a cell-specific manner. The Journal of steroid biochemistry and molecular biology. 1998;67(2):79-88.
135. Paech K, Webb P, Kuiper GG, Nilsson S, Gustafsson J-Å, Kushner PJ, et al. Differential ligand activation of estrogen receptors ERα and ERβ at AP1 sites. Science. 1997;277(5331):1508-10.
136. Webb P, Lopez GN, Uht RM, Kushner PJ. Tamoxifen activation of the estrogen receptor/AP-1 pathway: potential origin for the cell-specific estrogen-like effects of antiestrogens. Molecular Endocrinology. 1995;9(4):443-56.
137. McKay LI, Cidlowski JA. Molecular control of immune/inflammatory responses: interactions between nuclear factor-κB and steroid receptor-signaling pathways. Endocrine reviews. 1999;20(4):435-59.
138. Pfeilschifter J, Köditz R, Pfohl M, Schatz H. Changes in proinflammatory cytokine activity after menopause. Endocrine reviews. 2002;23(1):90-119.
139. Zwijsen RM, Wientjens E, Klompmaker R, van der Sman J, Bernards R, Michalides RJ. CDK-independent activation of estrogen receptor by cyclin D1. Cell. 1997;88(3):405-15.
140. Nilsson S, Mäkelä S, Treuter E, Tujague M, Thomsen J, Andersson G, et al. Mechanisms of estrogen action. Physiological reviews. 2001;81(4):1535-65.

141. Picard N, Charbonneau C, Sanchez M, Licznar A, Busson M, Lazennec G, et al. Phosphorylation of activation function-1 regulates proteasome-dependent nuclear mobility and E6-associated protein ubiquitin ligase recruitment to the estrogen receptor β. Molecular endocrinology. 2008;22(2):317-30.
142. Sauvé K, Lepage J, Sanchez M, Heveker N, Tremblay A. Positive feedback activation of estrogen receptors by the CXCL12-CXCR4 pathway. Cancer research. 2009;69(14):5793-800.
143. Pedram A, Razandi M, Levin ER. Nature of functional estrogen receptors at the plasma membrane. Molecular endocrinology. 2006;20(9):1996-2009.
144. Li L, Haynes MP, Bender JR. Plasma membrane localization and function of the estrogen receptor α variant (ER46) in human endothelial cells. Proceedings of the National Academy of Sciences. 2003;100(8):4807-12.
145. Doolan CM, Harvey BJ. A Gαs protein-coupled membrane receptor, distinct from the classical oestrogen receptor, transduces rapid effects of oestradiol on [Ca 2+] i in female rat distal colon. Molecular and cellular endocrinology. 2003;199(1):87-103.
146. Razandi M, Pedram A, Greene GL, Levin ER. Cell membrane and nuclear estrogen receptors (ERs) originate from a single transcript: studies of ERα and ERβ expressed in Chinese hamster ovary cells. Molecular Endocrinology. 1999;13(2):307-19.
147. Song RX, Barnes CJ, Zhang Z, Bao Y, Kumar R, Santen RJ. The role of Shc and insulin-like growth factor 1 receptor in mediating the translocation of estrogen receptor α to the plasma membrane. Proceedings of the National Academy of Sciences. 2004;101(7):2076-81.
148. Acconcia F, Ascenzi P, Bocedi A, Spisni E, Tomasi V, Trentalance A, et al. Palmitoylation-dependent estrogen receptor α membrane localization: regulation by 17β-estradiol. Molecular biology of the cell. 2005;16(1):231-7.
149. Simoncini T, Hafezi-Moghadam A, Brazil DP, Ley K, Chin WW, Liao JK. Interaction of oestrogen receptor with the regulatory subunit of phosphatidylinositol-3-OH kinase. Nature. 2000;407(6803):538-41.
150. Filardo EJ, Quinn JA, Bland KI, Frackelton Jr AR. Estrogen-induced activation of Erk-1 and Erk-2 requires the G protein-coupled receptor homolog, GPR30, and occurs via trans-activation of the epidermal growth factor receptor through release of HB-EGF. Molecular endocrinology. 2000;14(10):1649-60.
151. Stefano GB, Peter D. Cell surface estrogen receptors coupled to cNOS mediate immune and vascular tissue regulation: therapeutic implications. Medical Science Monitor. 2001;7(5):1066-74.
152. Benten WPM, Becker A, Schmitt-Wrede H-P, Wunderlich F. Developmental regulation of intracellular and surface androgen receptors in T cells. Steroids. 2002;67(11):925-31.
153. Simpkins JW, Yang S-H, Sarkar SN, Pearce V. Estrogen actions on mitochondria—physiological and pathological implications. Molecular and cellular endocrinology. 2008;290(1):51-9.
154. Scheller K, Sekeris CE. The effects of steroid hormones on the transcription of genes encoding enzymes of oxidative phosphorylation. Experimental physiology. 2003;88(1):129-40.
155. Bettini E, Maggi A. Estrogen induction of cytochrome c oxidase subunit III in rat hippocampus. Journal of neurochemistry. 1992;58(5):1923-9.
156. Van Itallie CM, Dannies PS. Estrogen induces accumulation of the mitochondrial ribonucleic acid for subunit II of cytochrome oxidase in pituitary tumor cells. Molecular Endocrinology. 1988;2(4):332-7.
157. Chen J-Q, Cammarata PR, Baines CP, Yager JD. Regulation of mitochondrial respiratory chain biogenesis by estrogens/estrogen receptors and physiological, pathological and

pharmacological implications. Biochimica et Biophysica Acta (BBA)-Molecular Cell Research. 2009;1793(10):1540-70.
158. Sekeris CE. The mitochondrial genome: a possible primary site of action of steroid hormones. In vivo (Athens, Greece). 1990;4(5):317.
159. Demonacos CV, Karayanni N, Hatzoglou E, Tsiriyiotis C, Spandidos DA, Sekeris CE. Mitochondrial genes as sites of primary action of steroid hormones. Steroids. 1996;61(4):226-32.
160. Chen JQ, Eshete M, Alworth WL, Yager JD. Binding of MCF-7 cell mitochondrial proteins and recombinant human estrogen receptors α and β to human mitochondrial dna estrogen response elements. Journal of cellular biochemistry. 2004;93(2):358-73.
161. Chen J-Q, Russo PA, Cooke C, Russo IH, Russo J. ERβ shifts from mitochondria to nucleus during estrogen-induced neoplastic transformation of human breast epithelial cells and is involved in estrogen-induced synthesis of mitochondrial respiratory chain proteins. Biochimica et Biophysica Acta (BBA)-Molecular Cell Research. 2007;1773(12):1732-46.
162. Gergely P, Grossman C, Niland B, Puskas F, Neupane H, Allam F, et al. Mitochondrial hyperpolarization and ATP depletion in patients with systemic lupus erythematosus. Arthritis & Rheumatism. 2002;46(1):175-90.
163. Perl A, Gergely P, Nagy G, Koncz A, Banki K. Mitochondrial hyperpolarization: a checkpoint of T-cell life, death and autoimmunity. Trends in immunology. 2004;25(7):360-7.
164. Li Q, Ward J, Banerjee S, Perl A, editors. Prominent changes in expression of Ca2+ fluxing, mitochondrial electron transport, and antioxidant pathway genes in peripheral blood lymphocytes of patients with systemic lupus erythematosus. Arthritis and Rheumatism; 2005: WILEY-BLACKWELL 111 RIVER ST, HOBOKEN 07030-5774, NJ USA.
165. Vyshkina T, Sylvester A, Sadiq S, Bonilla E, Canter JA, Perl A, et al. Association of common mitochondrial DNA variants with multiple sclerosis and systemic lupus erythematosus. Clinical Immunology. 2008;129(1):31-5.
166. Zhu Y, Bian Z, Lu P, Karas RH, Bao L, Cox D, et al. Abnormal vascular function and hypertension in mice deficient in estrogen receptor β. Science. 2002;295(5554):505-8.
167. Hayashi T, Fukuto JM, Ignarro LJ, Chaudhuri G. Basal release of nitric oxide from aortic rings is greater in female rabbits than in male rabbits: implications for atherosclerosis. Proceedings of the National Academy of Sciences. 1992;89(23):11259-63.
168. Nigro D, Fortes Z, Scivotetto R, Carvalho M. Simultaneous release of endothelium-derived relaxing and contracting factors induced by noradrenaline in normotensive rats. General Pharmacology: The Vascular System. 1990;21(4):443-6.
169. Huang A, Sun D, Koller A, Kaley G. 17β-Estradiol restores endothelial nitric oxide release to shear stress in arterioles of male hypertensive rats. Circulation. 2000;101(1):94-100.
170. Caulin-Glaser T, Garcia-Cardena G, Sarrel P, Sessa WC, Bender JR. 17β-estradiol regulation of human endothelial cell basal nitric oxide release, independent of cytosolic Ca2+ mobilization. Circulation Research. 1997;81(5):885-92.
171. Chen Z, Yuhanna IS, Galcheva-Gargova Z, Karas RH, Mendelsohn ME, Shaul PW. Estrogen receptor α mediates the nongenomic activation of endothelial nitric oxide synthase by estrogen. The Journal of clinical investigation. 1999;103(3):401-6.
172. Costa S, Anversa P, Scavone C, Sucupira M, Scivoletto R, Nigro D, et al., editors. Nitric oxide synthase activity in microvessels of SHR and normotensive rats: effects of estrogen. Journal of Hypertension; 1998: LIPPINCOTT WILLIAMS & WILKINS 227 EAST WASHINGTON SQ, PHILADELPHIA, PA 19106 USA.

173. Hisamoto K, Ohmichi M, Kurachi H, Hayakawa J, Kanda Y, Nishio Y, et al. Estrogen induces the Akt-dependent activation of endothelial nitric-oxide synthase in vascular endothelial cells. Journal of Biological Chemistry. 2001;276(5):3459-67.
174. Jayachandran M, Hayashi T, Sumi D, Iguchi A, Miller VM. Temporal effects of 17β-estradiol on caveolin-1 mRNA and protein in bovine aortic endothelial cells. American Journal of Physiology-Heart and Circulatory Physiology. 2001;281(3):H1327-H33.
175. Simoncini T, Genazzani AR, Liao JK. Nongenomic mechanisms of endothelial nitric oxide synthase activation by the selective estrogen receptor modulator raloxifene. Circulation. 2002;105(11):1368-73.
176. Tolbert T, Thompson JA, Bouchard P, Oparil S. Estrogen-Induced Vasoprotection Is Independent of Inducible Nitric Oxide Synthase Expression Evidence From the Mouse Carotid Artery Ligation Model. Circulation. 2001;104(22):2740-5.
177. Cutolo M, Brizzolara R, Atzeni F, Capellino S, Straub RH, Puttini PCS. The immunomodulatory effects of estrogens. Annals of the New York Academy of Sciences. 2010;1193(1):36-42.
178. Jabbour HN, Sales KJ, Catalano RD, Norman JE. Inflammatory pathways in female reproductive health and disease. Reproduction. 2009;138(6):903-19.
179. Straub RH. The complex role of estrogens in inflammation. Endocrine reviews. 2007;28(5):521-74.
180. Leone M, Textoris J, Capo C, Mege J-L. Sex Hormones and Bacterial Infections. culture. 2012;15:100,000.
181. Druckmann R. Review: female sex hormones, autoimmune diseases and immune response. Gynecological Endocrinology. 2001;15(sup6):69-76.
182. Nalbandian G, Kovats S. Understanding sex biases in immunity. Immunologic research. 2005;31(2):91-106.
183. Klebanoff SJ. Estrogen binding by leukocytes during phagocytosis. The Journal of experimental medicine. 1977;145(4):983-98.
184. Bain BJ, England J. Normal haematological values: sex difference in neutrophil count. Br Med J. 1975;1(5953):306-9.
185. Bain BJ, England J. Variations in leucocyte count during menstrual cycle. Br Med J. 1975;2(5969):473-5.
186. Smith JM, Shen Z, Wira CR, Fanger MW, Shen L. Effects of menstrual cycle status and gender on human neutrophil phenotype. American Journal of Reproductive Immunology. 2007;58(2):111-9.
187. Mathur S, Mathur RS, Goust JM, Williamson HO, Fudenberg HH. Cyclic variations in white cell subpopulations in the human menstrual cycle: correlations with progesterone and estradiol. Clinical immunology and immunopathology. 1979;13(3):246-53.
188. Molero L, Garcıa-Durán M, Diaz-Recasens J, Rico L, Casado S, López-Farré A. Expression of estrogen receptor subtypes and neuronal nitric oxide synthase in neutrophils from women and men. Cardiovascular research. 2002;56(1):43-51.
189. Stygar D, Masironi B, Eriksson H, Sahlin L. Studies on estrogen receptor (ER) α and β responses on gene regulation in peripheral blood leukocytes in vivo using selective ER agonists. Journal of endocrinology. 2007;194(1):101-19.
190. García-Durán M, de Frutos T, Díaz-Recasens J, García-Gálvez G, Jiménez A, Montón M, et al. Estrogen stimulates neuronal nitric oxide synthase protein expression in human neutrophils. Circulation research. 1999;85(11):1020-6.

191. Molloy EJ, O'Neill AJ, Grantham JJ, Sheridan-Pereira M, Fitzpatrick JM, Webb DW, et al. Sex-specific alterations in neutrophil apoptosis: the role of estradiol and progesterone. Blood. 2003;102(7):2653-9.
192. Curran EM, Berghaus LJ, Vernetti NJ, Saporita AJ, Lubahn DB, Estes DM. Natural killer cells express estrogen receptor-α and estrogen receptor-β and can respond to estrogen via a non-estrogen receptor-α-mediated pathway. Cellular immunology. 2001;214(1):12-20.
193. Shim GJ, Gherman D, Kim HJ, Omoto Y, Iwase H, Bouton D, et al. Differential expression of oestrogen receptors in human secondary lymphoid tissues. The Journal of pathology. 2006;208(3):408-14.
194. Hao S, Zhao J, Zhou J, Zhao S, Hu Y, Hou Y. Modulation of 17β-estradiol on the number and cytotoxicity of NK cells in vivo related to MCM and activating receptors. International immunopharmacology. 2007;7(13):1765-75.
195. Hao S, Li P, Zhao J, Hu Y, Hou Y. 17 [beta]-Estradiol Suppresses Cytotoxicity and Proliferative Capacity of Murine Splenic NK1. 1+ Cells. Cellular & molecular immunology. 2008;5(5):357.
196. Yovel G, Shakhar K, Ben-Eliyahu S. The effects of sex, menstrual cycle, and oral contraceptives on the number and activity of natural killer cells. Gynecologic oncology. 2001;81(2):254-62.
197. Henderson TA, Saunders PT, Moffett-King A, Groome NP, Critchley HO. Steroid receptor expression in uterine natural killer cells. The Journal of Clinical Endocrinology & Metabolism. 2003;88(1):440-9.